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Synthesis and evaluation of acylguanidine FXa inhibitors

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ABSTRACT

A series of acylguanidine derivatives were prepared and investigated as inhibitors of Factor Xa (FXa). These compounds were made by guanidine acylation with carboxylic acids using carbonyl diimidazole (CDI) as the coupling reagent. Conditions for the rapid synthesis and purification of these compounds are described along with their ability to inhibit FXa. The best FXa inhibitor is **1** with a FXa IC_{50} of 6 nM.

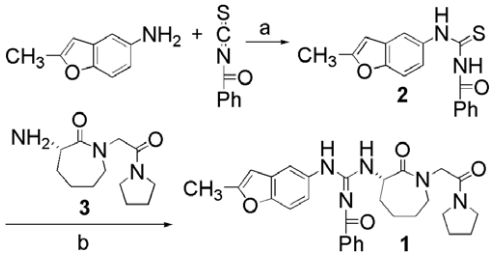
Heparin and Coumadin® are two of the most commonly administered anticoagulants. However, heparin must be used intravenously, and both heparin and Coumadin® can lead to excessive bleeding and hemorrhage. A safer and more convenient oral anticoagulant therapy is needed that would alleviate these liabilities within the current treatment regimens.[1] At the junction of the intrinsic and extrinsic (tissue factor) coagulation cascades lies the trypsin-like serine protease factor Xa (FXa).[2] FXa inhibition offers a reduced risk of unwarranted bleeding compared with thrombin inhibitors.[3] Inhibition of FXa has been an active area of pharmaceutical development with many intravenous[4] and orally active compounds[5] currently under investigation such as the parenteral inhibitor fondaparinux[6] and the small molecule inhibitors razaxaban,[7] apixaban,[8] rivaroxaban (BAY 59-7939),[9] and LY-517717.[10]

Earlier disclosures from these laboratories have elaborated a series of lactam-based urea, thiourea, carbamate, amide, and sulfonamide derivatives[11] as well as ketene aminal analogs.[12] We report herein the activity of a series of analogous lactam-based acylguanidine derivatives as well as a method for their rapid synthesis and purification from simple guanidine precursors.

Caprolactam pyrrolidine amide **3**[11] and 5-amino-2-methylbenzofuran[12] have been described previously as providing potent FXa inhibitors when linked as ureas and ketene aminals. We created a potent, new chemotype by linking these groups through an acylated guanidine **1** (IC_{50} = 6 nM), whose initial synthesis is shown in Scheme 1.

The synthesis of **1** began by coupling 5-amino-2-methylbenzofuran with benzoyl isothiocyanate in chloroform (81% yield). The product acylthiourea was then coupled with aminocaprolactam compound **3** using EDCI to provide **1** (10% yield). While this was a simple process, the low yield in the coupling step, albeit unoptimized, as well as the limited availability of diverse acyl isothiocyanates precluded this approach for the rapid synthesis of a large number of analogs. As we desired to explore diversity in the acyl substituent using various aryl groups as replacements for the 2-methylbenzofuran fragment,[13] an alternative route to compounds such as **6** was needed.

A more versatile route to acylguanidines is shown in Scheme 2. An aryl amine can be converted to the corresponding isothiocyanate, coupled with aminolactam **3** to yield thiourea **4**, aminated to guanidine **5**, and then acylated with a carboxylic acid to provide



Scheme 1. Reagents and conditions: (a) $CHCl_3$, rt, 81%; (b) EDCI, DMF/$CHCl_3$, rt, 10%.

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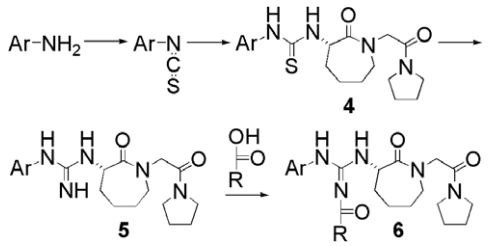
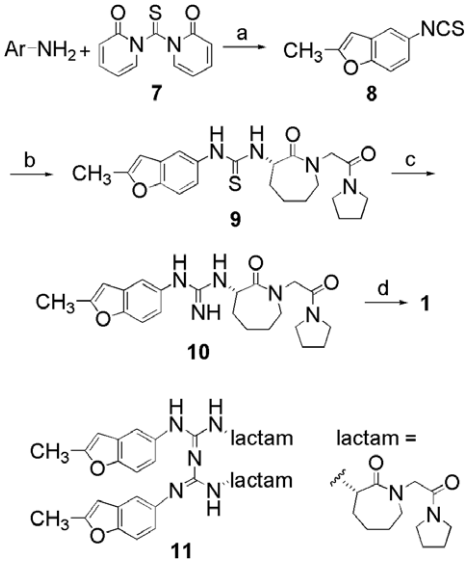


Scheme 2. General synthesis of acyl guanidines through isothiocyanate, thiourea, and guanidine.

the product **6**. While this was a four-step process, the last step was the key reaction, allowing a large number of products to be produced through the final combination of guanidines and carboxylic acids. Thus, we chose to optimize the chemistry in this route, particularly the last step, and to devise a fast and simple method for the purification of the final products. In order to establish the regioselectivity of the guanidine acylation, we optimized the route with compound **1** as the target, because the position of the acyl group had been unambiguously established through the isothiocyanate route described in Scheme 1.

Executing the general synthesis outlined in Scheme 2, 5-amino-2-methylbenzofuran was reacted with commercially available 1,1′-thiocarbonyldi-2(1*H*)-pyridone (**7**) to provide the corresponding isothiocyanate **8** (91% yield, Scheme 3).[14] The coupling of the isothiocyanate with caprolactam amine **3** gave thiourea **9** in 87% yield. Conversion to guanidine **10** was carried out using red mercury (II) oxide and 7 M ammonia in methanol (74%).[15] While 2 M ammonia in methanol also provided product **10**, we had observed up to 30% of a by-product having a molecular weight corresponding to the dimeric compound **11**. We reasoned that such a compound might arise by coupling between the product guanidine and unreacted thiourea **9**. As such, we were gratified to find that the use of higher ammonia concentrations (7 M) reduced the level of this by-product to less than 10%. With a reliable method in hand to make a variety of guanidines, we were now prepared to investigate the final acylation and purification sequence.



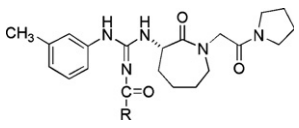
Scheme 3. Reagents and conditions: (a) CH_2Cl_2, rt, 91%; (b) **3** (1.1 equiv), $CHCl_3$, 60 °C, 87%; (c) red HgO (10 equiv), 7 M NH_3/CH_3OH, rt, 74%; (d) $PhCO_2H$ (1.5 equiv), CDI (1.5 equiv), CH_3CN, rt, 68%.

A brief selection of acylation conditions (acid chloride; PyBop/Et_3N; CDI)[16] suggested that the use of CDI[17] was superior, based on higher conversion and ease of purification. The acid chloride method produced undesired by-products (mono- and di-acylated species), while the use of PyBop concommitantly generates a phosphoramide which was difficult to separate from the products. The only by-products for the CDI method were imidazole (neutral, pK_a 6.95[18]) and unreacted guanidine (basic) and carboxylic acid (acidic) precursors. The wide differences in polarity or pK_a's for these species suggested that a simple method of purification could be developed. In order to investigate this, we optimized the reaction conditions for quantitative conversion of guanidine **10** to product **1**: benzoic acid (1.5 equiv) was reacted with CDI (1.5 equiv) in acetonitrile to which was then added the guanidine **1** (1.0 equiv). This reaction mixture was then used to find a purification method that would be efficient (high yielding), effective (high purity products), and simple (high throughput).

Our initial attempts at purification using anion or cation exchange resins were not successful as imidazole could not be separated from the products.[19] Purification on silica gel gave variable results as different acylguanidine products displayed a wide range of polarities and elution profiles. However, the method that we used for the analysis of reaction progress, reverse phase C-18 (octadecyl-bound silica gel) liquid chromatography (LC), suggested a simple solution. All of the reaction components and by-products (benzoic acid, guanidine **10**, imidazole) eluted faster from the C-18 column than the product acylguanidine.[20] A low concentration (5–20%) of methanol or acetonitrile in water, when passed through a column of C-18, should elute all of the undesired components. Product **1** should not elute until a higher level of the organic component was used.[21] The reaction products proved to be suitable for step-gradient purification on disposable C-18 sample cleanup cartridges.[22]

With a method in hand for the synthesis and rapid purification of acylguanidines of structure **6**, our next goal was to evaluate alternatives to the 2-methylbenzofuran portion of **1** while surveying the acyl component. The *m*-tolyl and *p*-anisyl analogs were chosen due to the structural homology with compound **1**. A number of carboxylic acids were selected as coupling components, and representative groups are shown in Tables 1 and 2.[24]

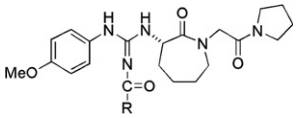
Table 1
m-Tolyl acylguanidines



Compound #	R	FXa IC_{50}[a] (nM)	Yield (%)	Purity (%)
12	Ph	245	85	87
13	3-MeO-Ph	276	35	96
14	4-MeO-Ph	709	44	89
15	4-Me_2N-Ph	517	92	90
16	3-Cl-Ph	371	55	91
17	4-Cl-Ph	198	62	91
18	3,5-Di-Cl-Ph	835	25	94
19	3-F-Ph	373	63	93
20	4-F-Ph	222	74	94
21	3,4-Di-F-Ph	271	35	88
22	3,5-Di-F-Ph	396	50	94
23	3,4-Di-MeO-Ph	400	49	100
24	3,5-Di-MeO-Ph	1159	51	88

[a] IC_{50} measured against human FXa using the cleavage of synthetic substrate S-2222.

Table 2
p-Anisyl acylguanidines



Compound #	R	FXa IC_{50}[a] (nM)	Yield (%)	Purity (%)
25	Ph	246	71	97
26	3-MeO-Ph	193	69	96
27	4-MeO-Ph	227	74	97
28	4-Me_2N-Ph	403	52	98
29	3-Cl-Ph	267	65	97
30	4-Cl-Ph	294	58	93
31	3,5-Di-Cl-Ph	546	40	92
32	3-F-Ph	256	53	91
33	4-F-Ph	368	54	95
34	3,4-Di-F-Ph	272	42	85
35	3,5-Di-F-Ph	208	25	82
36	3,4-Di-MeO-Ph	494	42	95
37	3,5-Di-MeO-Ph	872	66	83

[a] IC_{50} measured against human FXa using the cleavage of synthetic substrate S-2222.

The yields ranged from a modest 25% to 92% with purity levels of 82–100%. In spite of some low yields, this represented a method for rapid synthesis and purification of acylguanidines with good to excellent levels of product purity. Some features worth noting are (1) the simple 'on–off' cartridge purification that was used,[25] (2) minimal reaction optimization, and (3) significant quantities (10–60 mg) of reasonably pure compounds were obtained for evaluation.

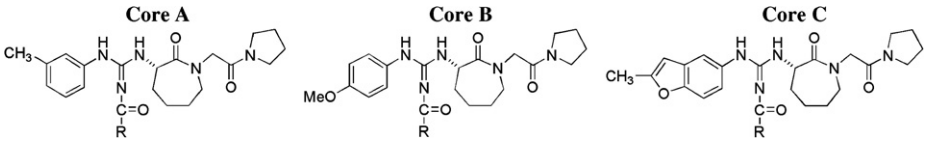
The compounds in Tables 1 and 2 showed moderate FXa inhibitory effects. Monosubstituted benzoic acids in general provided more potent acylguanidine products than disubstituted analogs; however, disubstitution with fluorine was better tolerated than with chlorine or methoxy. The most apparent trend was a significant drop in potency of the *m*-tolyl **12** (245 nM) and *p*-anisyl **25** (246 nM) analogs when compared to the corresponding 2-methylbenzofuran-5-yl compound **1** (6 nM). Within the set of carboxylic acids examined, all of the compounds in Tables 1 and 2 were nearly two orders of magnitude less potent than compound **1**.

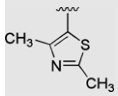
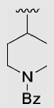
For all of the compounds in Tables 1 and 2 the concentration required to double the prothrombin-based clotting time in human plasma (EC_{2XPT}) was greater than 50 μM. This value for the original lead **1** was only marginally better (EC_{2XPT} = 48 μM). In a further effort to improve the potency of the *m*-tolyl and *p*-anisyl analogs relative to the 2-methylbenzofuran-5-yl compounds, as well as to improve the prothrombin time effects, we choose a small set of three additional carboxylic acids to examine. Two heterocyclic acids (isoxazole-5-carboxylic acid and 2,4-dimethylthiazole-5-carboxylic acid) and an amide containing compound (1-benzoylpiperidine-4-carboxylic acid) were selected. These compounds were chosen to assess the effects of heterocycles and an amide functional group.

The synthesis and in vitro results for these compounds are shown in Table 3. As expected, modest to good yields (19–90%) were obtained with good levels of purity (87–100%). However, continuing the trend observed above, the 2-methylbenzofuran-5-yl analogs **40**/**43**/**46** were significantly more potent (10–17 nM) than the *m*-tolyl compounds **38**/**41**/**44** (462–1073 nM) and*p*-anisyl compounds **39**/**42**/**45** (380–506 nM). In addition, while the *p*-anisyl compounds **39** and **45** exhibited very modest activity at best in the prothrombin assay (EC_{2XPT} = 45 and 48 μM, respectively), the 2-methylbenzofuran-5-yl analogs **40** (EC_{2XPT} = 17.7 μM), **43** (EC_{2XPT} = 42 μM), and **46** (EC_{2XPT} = 7.5 μM), all had superior activity. Relative to lead **1** (EC_{2XPT} = 48 μM), amide **46** was sixfold more potent in the prothrombin assay suggesting that polarity in this region of the molecule may improve in vivo potency.

In summary, we have developed a simple method for the efficient synthesis and purification of acylguanidines from carboxylic acid and guanidine precursors. The use of CDI as coupling reagent and a reverse phase cartridge purification strategy were key features that provided a rapid means for

Table 3
Comparison of selected acylguanidines



R	Core	Compound	FXa IC_{50}[a] (nM)	EC_{2XPT} (μM)	Yield (%)	Purity (%)
	**A**	**38**	628	50	51	87
	B	**39**	380	45	44	98
	C	**40**	14	17.7	90	97
	**A**	**41**	462	>50	44	98
	B	**42**	386	>50	41	97
	C	**43**	17	42	28	94
	**A**	**44**	1073	>50	67	98
	B	**45**	506	48	76	98
	C	**46**	10	7.5	19	100

[a] IC_{50} measured against human FXa using the cleavage of synthetic substrate S-2222.

obtaining products with good levels of purity. The FXa inhibitory potential of these compounds revealed a strong preference for the 2-methylbenzofuran in **1** (IC_{50} = 6 nM) relative to the *m*-tolyl and *p*-anisyl portions of **12** (IC_{50} = 245 nM) and **25** (IC_{50} = 246 nM), respectively. However, while the intrinsic potency of compound **1** was good, the concentration required to double the prothrombin-based clotting time in human plasma was low (EC_{2XPT} = 48 μM). Compound **46** (IC_{50} = 10 nM; EC_{2XPT} = 7.5 μM) displayed good intrinsic potency with a significantly improved prothrombin time. Further elaboration of this chemotype will be disclosed in a more detailed publication in the future.

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16. Multiple acylation products were observed with benzoyl chloride and we experienced difficulty in separating the PyBop-derived phosphoryl triamide by-products from the desired acylguanidines without resorting to preparative HPLC.
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19. Since the products were neutral and the potential impurities were either charged or very polar, we first examined ion exchange as a means of purification. While it was possible to remove the acidic unreacted carboxylic acid using an anion-exchange cartridge and the basic unreacted guanidine could be eliminated with a cation-exchange cartridge, the neutral by-product imidazole (pK_a 6.95) was problematic. This contaminant co-eluted with product from both types of ion-exchange cartridges. In addition, silica gel did not provide a general method of purification as the products had a range of polarities and required tedious adjustment of the solvent composition for efficient elution.
20. (a) The conditions for LC analysis were either (a) or (b): (a) YMC S5 ODS 4.6 × 50 mm column; gradient from 10% MeOH/0.1% aqueous TFA to 90% MeOH/0.1% aqueous TFA over 4 min; flow rate = 4 mL/min; 220 nM detection.; (b) Phenomenex Luna 5μ C-18 4.6 × 50 mm column; gradient from 10% MeOH/0.2% aqueous H_3PO_4 to 90% MeOH/0.2% aqueous H_3PO_4 over 4 min; flow rate = 4 mL/min; 220 nM detection.
21. An excess of benzoic acid and CDI was used to drive all of the guanidines to product ensuring that the reaction mixture would contain, after an aqueous quench, only the fast eluting benzoic acid and imidazole along with the less polar product.
22. Optimized conditions developed for the final reaction and purification steps were as follows: to benzoic acid (18 mg, 0.15 mmol, 1.5 equiv) in 0.4 mL of acetonitrile was added CDI (24 mg, 0.15 mmol, 1.5 equiv). After stirring at room temperature for 5 min, compound 10 (41 mg, 0.10 mmol) was added and the reaction progress monitored by LC. Within 2 h, the reaction was complete. After quenching with 1 mL of water, the resulting slurry was placed directly onto a 2 g C-18 cartridge[23] and eluted with 40 mL of 20% acetonitrile in water. The cartridge was then eluted with 5 mL of acetonitrile to yield, after concentration, product **1** (35 mg, 0.068 mmol, 68%) as a tan foam in 96% purity by LC analysis. This material was identical by ^1H and ^{13}C NMR, and co-eluted on LC, LC-MS, and TLC with compound **1** produced by the alternative method shown in Scheme 1.
23. The cartridges were first washed sequentially with 4–5 vol each of methanol then water. Reaction mixtures were loaded onto the cartridges under water. We have achieved comparable results using Varian 2 g MegaBondElut C18 HF cartridges (cat. #14256015), United Chemical Technologies 2.5 g Endcapped C-18 cartridges (cat. #CEC181(2500)6), or cartridges prepared with 2 g of J.T. Baker Bakerbond C-18 40 μM Prep LC Packing (cat. #7025-01).
24. A modification of the reaction conditions described above was made when synthesizing multiple acylguanidines simultaneously: the carboxylic acid (1.5 equiv) and CDI (1.5 equiv) were combined and allowed to react for 2 h. The guanidine (1.0 equiv) was then added and the reaction mixture was stirred for 16 h.
25. We viewed this as an 'on–off' cartridge purification strategy whereby the product would remain 'on' the C-18 packing with a high concentration of water, while the undesired components would elute 'off'. The concentration of the organic component in the solvent could then be increased to elute the product.